

Applicant: SCARINGE, Stephen  
Serial No.: 10/635,108  
Filing Date: August 5, 2003  
Amendment and Reply to Nonfinal Office Action  
September 27, 2004  
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**Amendments to the Specification:**

Please replace paragraph 0034 with the following amended paragraph:

An shRNA can be prepared by separately synthesizing each of the oligonucleotides X<sub>1</sub> and X<sub>2</sub> and then coupling the oligonucleotides together as a single hairpin by conjugation to each end of a separately prepared flexible linker. Alternatively, a shRNA can be prepared by the phosphoramidite method described by Beaucage and Caruthers (*Tetrahedron Lett.*, (1981) 22:1859-1862), or by the triester method according to Matteucci, et al., (*J. Am. Chem. Soc.*, (1981) 103:3185), ~~each of which is specifically incorporated herein by reference~~, or by other chemical methods using a commercial automated oligonucleotide synthesizer.

Please replace paragraph 0056 with the following amended paragraph:

Oligonucleotide synthesis conditions were adapted from U.S. Patent No. 5,889,136, column 7 to column 15, to Scaringe and Caruthers, which is specifically incorporated herein by reference. The hairpins and control duplexes were synthesized on a 0.2 mmol dT column using 5'-silyl-2'-ACE chemistry on a 394 ABI instrument according to the method described in U.S. Patent No. 6,111,086 to Scaringe, column 3, line 18 to column 12, line 37, which is specifically incorporated herein by reference. The protocols can be adapted by those skilled in the art to any commercially available synthesizer. Following synthesis on the synthesizer, the polymer support is treated with a 1M solution of disodium 2-carbamoyl-2-cyanoethylene-1,1-dithiolate·trihydrate (S<sub>2</sub>Na<sub>2</sub>) to remove the methyl protecting groups from the phosphates. The S<sub>2</sub>Na<sub>2</sub> reagent was washed out with water and acetone. The dried support was treated with 40% N-methylamine in water at 55°C to cleave all base-labile protecting groups and release the oligonucleotide into solution. The oligonucleotides were brought up to a volume 1.6 mL with sterile water. Two aliquots were taken for a quantity and quality assay. For quantity, a 1:100 dilution was used to read the Optical Density Units. For quality, the product was analyzed under highly denaturing conditions using polyacrylamide gel

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electrophoresis (PAGE) with 7M urea at 60° C. A 10 mL 2'-ACE protected RNA aliquot was electrophoresed on a 15% polyacrylamide gel. The gel was run at 40° C for approximately 4 hours.